THE DEVELOPMENT OF HALF AND QUARTER EGGS OF ARBACIA PUNCTULATA AND OF STRONGLY CENTRIFUGED WHOLE EGGS

ETHEL BROWNE HARVEY

(*From the Marine Biological Laboratory, Woods Hole, and Washington Square College, New York University*)

When the unfertilized eggs of *Arbacia punctulata* are strongly centrifuged in a sugar solution of the same (or graded) density, they become dumb-bell shaped and then separate into two nearly equal spheres or half-eggs, one colorless and the other pigmented (E. N. Harvey, 1931). Further, when the colorless spheres are again centrifuged, more strongly, in a sugar solution of the same (or graded) density, they separate into two spheres or quarter-eggs, one without visible granules and the other with granules. All of these half- and quarter-eggs, as well as the deformed total eggs just before pulling apart, can be fertilized and develop. We can in this way obtain "egg fragments" of very definite size and content in great numbers. This obviates the former tedious method of cutting individual eggs with a glass needle (Harnley, 1926; Hörstadius, 1928; Plough, 1929; Tennent, Taylor and Whitaker, 1929; Whitaker, 1929) and insures a much more accurate division of the egg into parts of known structure than the old haphazard and harmful method of shaking the eggs into pieces (Hertwigs, Boveri, Driesch, Morgan, etc.).[1] Sea urchin eggs centrifuged in sea water alone do not separate into spheres owing to the fact that they sink to the bottom and are crushed by the force exerted on them. The method employed was to put two parts 0.95 molal sugar (95 per cent of 342 grams cane sugar added to 1 liter tap water, an isotonic solution) in the centrifuge tubes and above this one part of sea water containing eggs; the tubes were rolled gently to obtain partial mixing and then centrifuged at about 7000 r.p.m. (11 cm. radius) for four minutes. On removal of the tubes, the colorless spheres formed a whitish layer just beneath the surface, the whole eggs, which had not broken apart, formed a reddish layer about halfway down the tube and the pigmented spheres rested at the bottom of the tubes. One could then pipette off any one of the three kinds of eggs with practically no admixture of the other varieties. The sugar solution was found to have no ill effect on the eggs, since eggs could be kept in this solution for at least five hours

[1] The centrifuge method of separating eggs supplements rather than supplants the cutting method, since the granular constitution of fragments is different in the two cases.

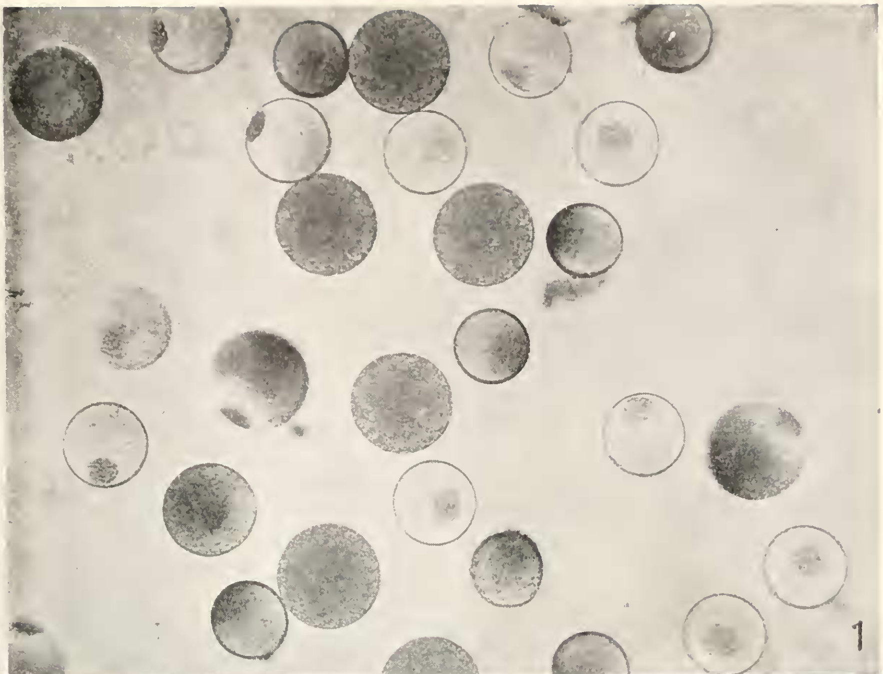
and when returned to sea water could be fertilized, and developed normally. Eggs cannot be fertilized, however, while in the sugar solution although the sperms are active and surround the egg. The rapid centrifuging also has no ill effect upon the eggs, as eggs subjected to this treatment develop into normal plutei when fertilized in sea water. To separate the colorless half-eggs into two parts, they were taken from the centrifuge tubes and put into other centrifuge tubes above a small amount of cane sugar solution (3 parts of 0.95 molal sugar to 1 part sea water) and a little sea water placed above. The tubes were then centrifuged for 45 minutes at about 10,000 r.p.m. (radius 11 cm.)

Unfertilized Whole, Half- and Quarter-eggs

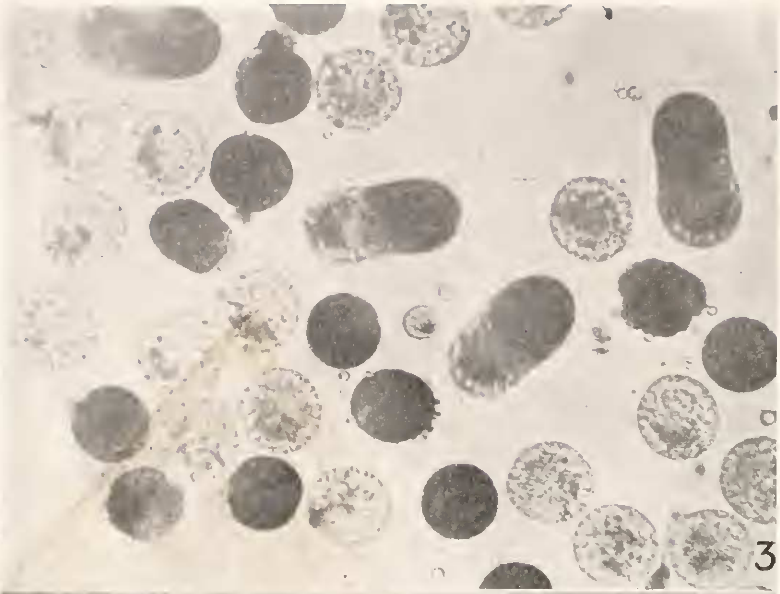
When normal *Arbacia* eggs are centrifuged as described above, they become stratified while still spherical into five layers, (1) oil on top, (2) a clear layer without visible (*i.e.*, under ordinary illumination) granules, in which lies the nucleus, (3) a thin granular layer separated out only with strong centrifugal force, and not heretofore described and which we have, therefore, termed "the fifth layer," (4) a large yellowish yolk layer, and (5) a layer of red pigment concentrated at the heavier pole (Fig. 25). The fifth layer can be very beautifully demonstrated by staining with methyl violet or methyl green when it becomes purple, or with Janus green when it becomes green.

The eggs after stratification become elongate, then dumb-bell shaped (Fig. 25) and then break into two slightly unequal spheres, usually through the upper part of the yolk. The colorless sphere (cf. Fig. 1), slightly larger, contains therefore the oil, below which always lies the nucleus; the clear layer, without visible granules; the granular or fifth layer; and a little yolk at the heavier pole. The pigmented sphere, (cf. Fig. 13) slightly smaller, contains usually only yolk and pigment, the pigment being massed at the heavier pole; there is, of course, no nucleus in this sphere. In a few batches of eggs, the two spheres were almost equal in size, the separation having taken place in the fifth layer, a little of which appeared as a light cap on the pigmented sphere. Usually, however, there is quite an appreciable difference in size of the two half-eggs, and their relative size is fairly constant in any one centrifuged lot. When the colorless spheres are centrifuged again, as described above, they separate into (1) a larger sphere with oil, nucleus and clear layer—this quarter-egg now having practically no visible granules (Fig. 38); and (2) a quite small sphere composed entirely of the granules of the fifth layer and yolk, about half of each (cf. Fig. 46).

The average measurements obtained in typical lots of eggs are given in Table I. A photograph of unfertilized whole eggs and the two



Photograph 1. Unfertilized whole and half-eggs.
Photograph 2. 2 and 4-cell whole and colorless half-eggs.



Photograph 3. Blastulae of whole and colorless half-eggs.
Photograph 4. Five types of eggs, whole, half- and quarter-eggs.

kinds of half-eggs is given in Photograph 1. Photograph 4 is a picture of the five types of eggs, whole, half- and quarter-eggs.

Development of Half-eggs

Both the colorless and pigmented half-eggs can be fertilized in sea water immediately after centrifuging or at any later time. They both form fertilization membranes at the same time as the whole egg. These are often well separated from the surface of the egg but sometimes, especially in the pigmented spheres, rather closely investing. There is in both half-eggs a well marked ectoplasmic or hyaloplasmic layer formed on fertilization. The development of the two half-eggs must now be followed separately. All observations were made on living material, and the times given are for 23° C., when 50 per cent normal eggs cleave in 50 minutes.

The development of the colorless half-egg is quite normal. Many of the nuclear phenomena accompanying fertilization and cleavage can be seen with great clearness, and they parallel those described by

TABLE I

	Diameter	Volume	Sums of Volumes
	μ	μ^3	μ^3
Whole Egg	73	203700	
Colorless Half Egg	60	113100	205050
Pigmented Half Egg	56	91950	
Clear Quarter Egg	53	77950	111450
Granular Quarter Egg	40	33500	

Wilson (1895) in the living *Toxopneustes* egg, which is devoid of pigment and quite transparent; these phenomena cannot be observed in the normal living *Arbacia* egg on account of the pigment. The sperm aster can be seen in the granular area a few minutes after fertilization, and its approach toward the female pronucleus can be followed (Fig. 2). The female pronucleus travels down from its position under the oil cap toward the center of the egg about eight minutes after fertilization (Fig. 2), and as it moves down some of the oil spherules also move down from the oil cap. After the union of the pronuclei (Fig. 3) astral radiations extend through the granular area; in fact all the granules are often arranged in rays extending in a half circle from the nucleus (Fig. 4); there is no indication of rays (in the living egg) in the clear area free of granules. In the normal uncentrifuged egg at this period, the astral radiations extend throughout the cell, this being the "monaster stage" (Fig. 36). These radiations gradually fade out and the nucleus enlarges from a diameter of 12 μ to 16 μ (Fig. 5). This is the "streak stage" of the normal *Arbacia* egg, lasting from 20 to 40 minutes after fertilization, characterized by a curved band extending on either side of the nucleus (Fig. 37); this stage is referred to by Wilson in the *Toxopneustes* egg as the "pause." The nuclear wall now breaks down (Fig. 6) and soon afterwards there are again radiations, now from the two poles of the amphiaster, but present only in the granular zone (Figs. 7, 8). The half-eggs divide about the same time as normal control eggs, or sometimes a little earlier as Whitaker (1929) found for his diploid fragments; the plane of cleavage usually comes in perpendicular to the stratification (Fig. 9), but sometimes parallel with it (Fig. 10) or in any intermediate position, but it divides the egg into two equal parts (photograph 2) as in the normal egg. The following cleavages come in at right angles to the preceding and divide the blastomeres equally (Figs. 11, 12). Blastulae (photograph 3) are formed from the half-eggs, quite normal except for size and coloring. In many of the cultures the larvae remained for several days as very actively swimming blastulae; in some they developed into gastrulae and in some into plutei with well developed skeleton and arms exactly like the normal ones except that they were colorless and only half the size.

The pigmented spheres have no nucleus at the time of fertilization. The aster accompanying the sperm nucleus may very often be seen in the yolk 15–20 minutes after the fertilization membrane has been given off (Fig. 13). The nucleus, at first very small, enlarges and is quite noticeable about 30 minutes after fertilization (Fig. 14), and about the time of first cleavage of the whole and colorless half-eggs. This single nucleus now usually without radiations, after enlarging considerably (Fig. 15), breaks down about one hour after fertilization (Fig. 16). At about 80 minutes after fertilization (Fig. 19), when the total and colorless spheres are in the 4-cell stage, many of the pigmented spheres have two nuclei; either a dumb-bell shaped nucleus or a very small amphiaster can sometimes be seen preceding the binucleate stage (Figs. 17, 18). Usually no cleavage plane comes in, probably owing to the mechanical difficulty in cutting through the dense material. After several successive divisions of the nuclei, the pigmented half-eggs appear as reddish spheres containing many white circles, some with radiations, giving somewhat the appearance of pictures of the moon with craters on the surface (Fig. 20). There are often slight indications of cleavage planes running in from the periphery as indentations or notches (Figs. 21, 22). Rather rarely, the division planes come in quite normally and the egg divides into 2, 4 and 8 equal blastomeres

(Figs. 23, 24). The first cleavage plane in these cases may come in in any relation to the stratification, and the following planes at right angles to the preceding. Although it is difficult to determine in living material, it would seem that when cleavage planes do not come in at first, they come in later on after some 20 or 30 nuclei are formed, for the blastulae seem quite normal and appear, as far as I could tell, multicellular. The blastulae are quite active, but are not very viable, and relatively few in any batch develop much further. I have, however, had a number of quite normal gastrulae and some plutei with skeletons, but only two with well developed arms. None have survived more than nine days. There seems no doubt that these merogonic eggs *can* give rise to dwarf embryos similar to normal ones except for containing more pigment. Whether the failure of the majority of these eggs to develop far is due to lack of certain formative stuffs or to the lack of the female pronucleus, or to the over-crowding with dense material is not certain, but I should judge from the appearance and behavior of the developing eggs that the last explanation is the correct one. It may be, however, that only those half-eggs containing some of the fifth layer develop, but this awaits further investigation.

It would seem then that both half-eggs can develop into plutei, and that neither food material (or very little) nor a female pronucleus is necessary for development.

Development of Strongly Centrifuged Whole Eggs

When the unfertilized *Arbacia* eggs are strongly centrifuged, as mentioned before, they elongate, then become dumb-bell shaped before breaking apart (Fig. 25). When these are left either in the sugar solution or in sea water, they soon become oval and then spherical, often within an hour. But if they are removed to sea water and fertilized immediately, they retain their dumb-bell shape and the fertilization membrane follows the contour of the surface with usually a bulge at one or both poles. This must be due to a "setting" or gelation of the protoplasm following fertilization, for if the fertilization membranes are removed by drawing the eggs into a capillary pipette, the eggs retain their aspherical shape (and develop). This is another indication of increased viscosity following fertilization. One can often observe the male aster in the yolk or granular zone, and this apparently pulls some pigment granules along as it travels toward the center (Figs. 26, 27). The descent of the female pronucleus can be observed and the pull of the oil spheres toward the center, the union of the two pronuclei and the gradual fading out of the sperm aster whose radiations have been visible only in the granular zone (Fig. 27), the enlargement of the nucleus and its rupture (Figs. 29, 30). A very striking stage is shown in Fig. 28, a sharp line of demarcation running between the clear zone and the granular zone; this corresponds to the "streak" stage in the normal egg (Fig. 37). A streak is only rarely seen running perpendicular to the stratification in well centrifuged eggs, probably owing to the denseness of the material. Later, rays from the amphiaster are seen in the yolk and granules of the fifth layer, which by this time are thoroughly mixed (Fig. 31); either a whole aster whose mate is sometimes faintly distinguishable in the clear zone into which some granules have spread, or less commonly two half asters in the granular and yolk zone. It would seem that there must be a change of axis in the mitotic figure, for in normal eggs the long axis of the spindle is the same as the long axis of the "streak," whereas here it is usually perpendicular to it. The first cleavage plane comes in at almost the same time as in normal eggs, usually through or near the constriction and parallel (or at a slight angle) with the stratification, separating one colorless blastomere from the one containing yolk and pigment (Fig. 32). These two cells are usually unequal in size, more frequently the colorless cell is the smaller, though the two blastomeres are sometimes of size corresponding with the two half-eggs, the cleavage plane following the future separation plane (Photograph 2). The first cleavage plane comes in rarely in these elongated eggs perpendicular to the stratification, and this is sometimes followed by a second cleavage plane also along the long axis resulting in four sausage shaped cells. The second cleavage plane always comes in perpendicular to the first (Fig. 33, Photograph 2). Many years ago, Lyon (1907) and Morgan and Lyon (1907) found that the first cleavage plane in centrifuged *Arbacia* eggs was usually perpendicular to the stratification. The apparent contradiction is explained by the difference in shape of the eggs. In *elongated* eggs, the first cleavage plane comes in usually parallel with the stratification, in the short axis. In *spherical* eggs it comes in perpendicular with the stratification; this is true both for eggs which are spherical because not centrifuged sufficiently to become elongate, and for eggs which have been elongate but have resumed a spherical shape on standing in sea water before fertilization. In slightly elongate or oval shaped eggs, the first cleavage plane comes in one way or the other in about equal numbers.

The later cleavage planes often come in fairly regularly except that the clear cells often divide in advance of the pigmented cells, and are smaller (Fig. 34). Very frequently, the original first cleavage plane remains quite prominent, the first two blastomeres developing almost independently (Fig. 35). So much so, in fact, that double embryos are often produced, one colored and the other colorless, at first within the same membrane, and later swimming attached together. Slipper shaped blastulae (Photograph 3), normal gastrulae and plutei arise from the elongate and dumb-bell shaped eggs, but the stratification of pigment and yolk remains, and may be in any relation, apparently, to the axis of the embryo. Individual eggs have not been studied with reference to polarity.

Development of Quarter Eggs

The colorless half-eggs, when centrifuged again, are drawn out into dumb-bells (Fig. 51) and are then separated into spheres. The stratification is the same as before, the nucleus lying just below the oil cap in a large clear layer without visible granules; at the heavier pole is the granular or fifth layer and a layer of yolk. The half-egg breaks usually at the line between the clear layer and the granules, so that we obtain one perfectly clear sphere, with oil cap and nucleus (Fig. 38) and one granular sphere containing about an equal amount of granules (fifth layer) and yolk (cf. Fig. 46). This granular quarter-egg is much smaller than the clear quarter, about one sixth the volume of the whole egg.

Both of these quarter-eggs, as well as the dumb-bell shaped half-eggs can be fertilized in sea water, and throw off fertilization membranes. The ectoplasmic layer of the granular quarter is much thicker than that of the clear quarter, where it is thinner than in normal eggs.

Owing to the absence of granules in the clear quarter, nothing can usually be seen of the sperm aster; the female pronucleus can be observed migrating from the oil cap to the center of the egg about 25 minutes after fertilization, pulling some of the oil spheres along. This gradually enlarges from 12 μ to 16 μ, but no other change occurs for six hours or more (Fig. 39). In some eggs the nucleus becomes enormous, as large as 22 μ (nearly half the diameter of the egg, and an increase of six times in volume), but this is probably abnormal. The nucleus later breaks down and disappears (Fig. 40) and cleavage takes place some seven hours (or more) after fertilization (Figs. 41, 42) and in any plane with regard to the oil cap. The very slow cleavage of these diploid quarters is not in accord with Whitaker's (1929) explanation of cleavage rates. Other cleavages follow slowly, but usually the membrane breaks and there is a loose mass of cells, some perfectly clear and some with oil drops (Figs. 43, 45). I have obtained a few intact later cleavages, particularly in eggs left for several hours after recentrifuging before fertilizing them (Fig. 44).

The granular quarter-eggs (Fig. 46), on the other hand, although having no female nucleus, develop quite normally. The male aster can be seen in the granules (Fig. 47), the nucleus enlarges, disappears, and the egg cleaves a little later than the control eggs (Fig. 48). This then divides into 4, 8, 16 approximately equal cells usually retaining the fertilization membrane (Figs. 49, 50).

Whether the quarter-eggs could give rise to swimming plutei will be investigated further. This part of the work was done for a short period late in September when the eggs are not in the best condition. The granular quarters went as far as the stage just before they become free-swimming and looked quite normal at that time.

The recentrifuged half-eggs which have become dumb-bell shaped (Fig. 51) retain their shape if fertilized immediately, and round up if left unfertilized just as the whole eggs do. The rounded eggs develop in the same way as before recentrifuging. The dumb-bell shaped half-eggs form a fertilization membrane following their contour and the nuclear phenomena accompanying fertilization can be clearly seen (Figs. 52–54). The first cleavage usually comes in near the junction of the clear with the granular area, giving one clear cell with oil cap and one granular cell (Fig. 55). The granular cell often precedes the clear cell in division just as it does when completely separate. The fertilization membrane usually breaks after several divisions, giving a loose mass of cells, some clear and some granular (Figs. 56–59).

Micro-dissection

The difference in the material of the white and red half-eggs can be well demonstrated by micro-dissection. When the colorless half-eggs are punctured by a needle they immediately explode, the granules and nucleus flowing out and leaving the membrane empty. When the pigmented half-eggs are punctured, there is no flow of granules, the material is quite pliable and elastic; it can be pulled out in strands which will go back again and resume a spherical form, or it can be cut in parts, each of which may round up. The stratified whole egg responds in a similar way. When the clear zone is punctured, the granules flow out. When the yolk or red layers are punctured, the material can be pulled out and released without any explosion or loss of material, and it behaves like an elastic and pliable substance. When the clear quarter-egg is punctured, it explodes immediately. When the granular quarter is punctured, the granules flow out but quite slowly.

Parthenogenesis

The question of parthenogenesis in the half- and quarter-eggs has been studied only slightly. Just (1928), and others previously, found that by treating unfertilized *Arbacia* eggs for a few seconds with distilled water and then returning them to sea water, the eggs formed fertilization membranes and developed to the stage just before cleavage. When the half- and quarter-eggs are thus treated, they all form beautiful fertilization membranes and good ectoplasmic layers. In the centrifuged whole eggs and in colorless half-eggs, the nucleus descends toward the center of the egg just as in fertilized eggs; this then is *not* an attraction by the male pronucleus. The nucleus enlarges and breaks just as in fertilized eggs. Astral radiations characteristic of fertilized eggs at the time of union of the pronuclei are, of course, absent, but the astral radiations from the amphiaster are later seen in the granular zone. In the pigmented half-eggs, no development further than the formation of the fertilization membrane and ectoplasmic layer has been observed, nor would it be expected since there is no nucleus of any sort. The clear quarters start to develop just as the colorless half-eggs, as indicated by the descent of the nucleus to the center. The granular quarters, like the pigmented half-eggs, show no further development after the formation of the fertilization membrane and ectoplasmic layer.

Summary

1. With strong centrifugal force and the proper medium, *Arbacia* eggs can be separated into two half-eggs, one colorless containing oil, nucleus, clear layer, fifth (granular) layer and a little yolk; the other slightly smaller containing yolk and pigment. With greater centrifugal force, the colorless half-eggs can be separated into quarter-eggs, one perfectly clear with oil and nucleus; the other, smaller, with fifth layer (granules) and yolk. All of these half- and quarter-eggs can be fertilized, form fertilization membranes and cleave.

2. Nuclear phenomena accompanying fertilization and cleavage, quite normal, can be observed with great clearness in the colorless half-eggs. Astral rays occur only where granules are present. These half-eggs cleave regularly and form swimming blastulae and plutei, normal except for color and size.

3. The pigmented half-eggs develop with only the male nucleus which divides repeatedly, usually without cell division. Some blastulae and a few plutei developed but these eggs and larvae are not very viable.

4. Whole eggs, centrifuged till dumb-bell shaped, retain their shape if fertilized immediately, even if the fertilization membrane is removed. The first cleavage in elongate eggs is usually parallel with the stratification, in spherical eggs it is usually perpendicular to it. Slipper shaped blastulae develop from the elongate eggs, and normal plutei.

5. Clear quarter-eggs begin to cleave very slowly (after 7 hours), and usually form loose clusters of cells owing to the breaking of the fertilization membrane.

6. Granular quarter-eggs develop with only the male nucleus and a little more slowly than the normal whole eggs; cleavage is quite regular but no swimming blastulae were obtained.

7. The pigmented half-eggs can be drawn out with a microdissection needle, and the material is pliable and elastic; the colorless half-eggs explode when punctured, pouring out granules. The clear quarter-eggs collapse immediately when punctured, and the granular quarters pour out their granules slowly.

8. All of the half- and quarter-eggs will start to develop parthenogenetically, *i.e.*, throw off a fertilization membrane, if treated with distilled water. Only those with a nucleus develop further, till just before cleavage.

LITERATURE LIST

Harnley, M. H., 1926. Localization of the Micromere Material in the Cytoplasm of the Egg of Arbacia. *Jour. Exper. Zoöl.*, **45**: 319.

Harvey, E. N., 1931. The Tension at the Surface of Marine Eggs, especially those of the Sea Urchin, Arbacia. *Biol. Bull.*, **61**: 273.

Hörstadius, S., 1928. Über die Determination des Keimes bei Echinodermen. *Acta Zoologica*, **9**: 1.

Just, E. E., 1928. Initiation of Development in Arbacia. IV. pp. 1–191. *Protoplasma*, **5**: 97–126.

Lyon, E. P., 1907. Results of Centrifugalizing Eggs. *Arch. f. Entw. Mech.*, **23**: 151–173.

Morgan, T. H., and E. P. Lyon, 1907. The Relation of the Substances of the Egg, Separated by a Strong Centrifugal Force, to the Location of the Embryo. *Arch. f. Entw. Mech.*, **24**: 147–159.

Plough, H. H., 1929. Determination of Skeleton-forming Material at the Time of the First Cleavage in the Eggs of Echinus and Paracentrotus. *Arch. f. Entw. Mech.*, **115**: 380–395.

Tennent, D. H., C. V. Taylor, and D. M. Whitaker, 1929. An Investigation on Organization in a Sea Urchin Egg. *Carnegie Inst. Pub. Wash.*, No. 391, pp. 1–104.

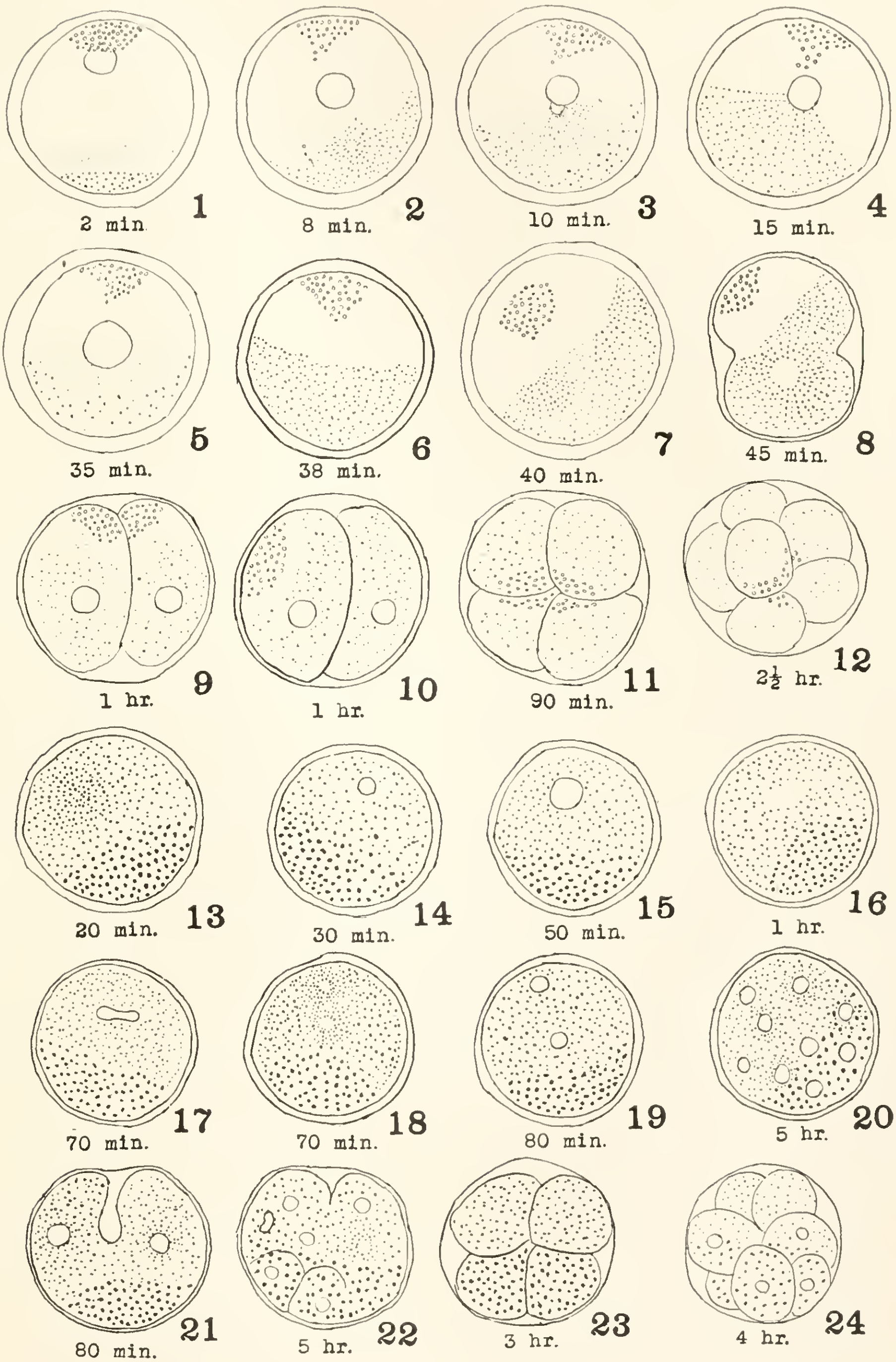
Whitaker, D. M., 1929. Cleavage Rates in Fragments of Centrifuged Arbacia Eggs. *Biol. Bull.*, **57**: 159–171.

Wilson, E. B., 1895. An Atlas of the Fertilization and Karyokinesis of the Ovum. New York.

DESCRIPTION OF PLATES

The drawings have been made from living eggs entirely, and are magnified 266 X. The large solid dots represent pigment, the coarse stippling yolk granules, fine stippling the granules of the fifth layer, small circles oil drops. The times given are times after fertilization, approximate for 23° C. (controls cleave in 50 minutes).

Figs. 1–12. Colorless half-eggs; Figs. 13–24 pigmented half-eggs; Figs. 25–35 centrifuged whole eggs; Figs. 36, 37 normal whole eggs; Figs. 38–45 clear quarter-eggs; Figs. 46–50 granular quarters; Figs. 51–59 recentrifuged colorless half-eggs.


2 min. 1
8 min. 2
10 min. 3
15 min. 4
35 min. 5
38 min. 6
40 min. 7
45 min. 8
1 hr. 9
1 hr. 10
90 min. 11
2½ hr. 12
20 min. 13
30 min. 14
50 min. 15
1 hr. 16
70 min. 17
70 min. 18
80 min. 19
5 hr. 20
80 min. 21
5 hr. 22
3 hr. 23
4 hr. 24